Limits:

– *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent, expressed as glyceryl trinitrate);
– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent, expressed as glyceryl trinitrate);
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

ASSAY

Test solution. Prepare a solution containing 1.0 mg of glyceryl trinitrate in 250.0 mL of *methanol R*.

Reference solution. Dissolve 70.0 mg of *sodium nitrite R* in *methanol R* and dilute to 250.0 mL with the same solvent. Dilute 5.0 mL of the solution to 500.0 mL with *methanol R*.

Into three 50 mL volumetric flasks introduce 10.0 mL of the test solution, 10.0 mL of the reference solution and 10 mL of *methanol R* as a blank. To each flask add 5 mL of *dilute sodium hydroxide solution R*, close the flask, mix and allow to stand at room temperature for 30 min. Add 10 mL of *sulfanilic acid solution R* and 10 mL of *dilute hydrochloric acid R* and mix. After exactly 4 min, add 10 mL of *naphthylethylenediamine dihydrochloride solution R*, dilute to volume with *water R* and mix. After 10 min read the absorbance (*2.2.25*) of the test solution and the reference solution at 540 nm using the blank solution as the compensation liquid.

Calculate the percentage content of glyceryl trinitrate using the following expression:

$$\frac{A_T \times m_S \times C}{A_R \times m_T \times 60.8}$$

A_T = absorption of the test solution;
m_T = mass of the substance to be examined, in milligrams;
C = percentage content of sodium nitrite used as reference;
A_R = absorption of the reference solution;
m_S = mass of sodium nitrite, in milligrams.

STORAGE

Store the diluted solutions (10 g/L) protected from light, at a temperature of 2 °C to 15 °C.

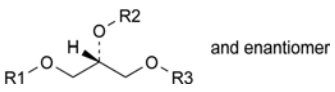
Store more concentrated solutions protected from light, at a temperature of 15 °C to 20 °C.

LABELLING

The label states the declared content of glyceryl trinitrate.

IMPURITIES

A. inorganic nitrates,



and enantiomer

B. R1 = NO_2, R2 = R3 = H: (2*RS*)-2,3-dihydroxypropyl nitrate,

C. R1 = R3 = H, R2 = NO_2: 2-hydroxy-1-(hydroxymethyl)ethyl nitrate,

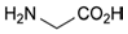
D. R1 = R2 = NO_2, R3 = H: (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate,

E. R1 = R3 = NO_2, R2 = H: 2-hydroxypropane-1,3-diyl dinitrate.

01/2008:0614
corrected 6.0

GLYCINE

Glycinum



$C_2H_5NO_2$ M_r 75.1
[56-40-6]

DEFINITION

2-Aminoacetic acid.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, very slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *glycine CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (60 per cent V/V) R*, evaporate to dryness and record the spectra again.

B. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results: the principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg in 5 mL of *water R*, add 1 mL of *strong sodium hypochlorite solution R* and boil for 2 min. Add 1 mL of *hydrochloric acid R* and boil for 4-5 min. Add 2 mL of *hydrochloric acid R* and 1 mL of a 20 g/L solution of *resorcinol R*, boil for 1 min and cool. Add 10 mL of *water R* and mix. To 5 mL of the solution add 6 mL of *dilute sodium hydroxide solution R*. The solution is violet with greenish-yellow fluorescence. After a few minutes, the colour becomes orange and then yellow and an intense fluorescence remains.

TESTS

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*): 5.9 to 6.4.

Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R*.

Ninhydrin-positive substances. Thin-layer chromatography (*2.2.27*).

Test solution (a). Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with *water R*.

Reference solution (a). Dissolve 10 mg of *glycine CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of test solution (a) to 200 mL with *water R*.

Reference solution (c). Dissolve 10 mg of *glycine CRS* and 10 mg of *alanine CRS* in *water R* and dilute to 25 mL with the same solvent.

Plate: *cellulose for chromatography R* as the coating substance.

Mobile phase: *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application: 5 µL.

Development: over 2/3 of the plate.

Drying: at 80 °C for 30 min.

Detection: spray with *ninhydrin solution R* and dry at 100-105 °C for 15 min.

System suitability: the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limits: in the chromatogram obtained with test solution (a):

– *any impurity*: any spots, apart from the principal spot, are not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*): maximum 75 ppm.

Dissolve 0.67 g in *water R* and dilute to 15 mL with the same solvent.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 mL of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

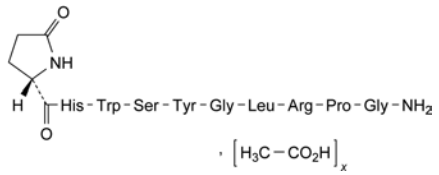
Dissolve 70.0 mg in 3 mL of *anhydrous formic acid R* and add 30 mL of *anhydrous acetic acid R*. Immediately after dissolution, titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 7.51 mg of $C_2H_5NO_2$.

01/2008:0827
corrected 7.0

GONADORELIN ACETATE

Gonadorelini acetas



$C_{55}H_{75}N_{17}O_{13},xC_2H_4O_2$ M_r 1182 ($C_{55}H_{75}N_{17}O_{13}$)
[34973-08-5]

DEFINITION

Gonadorelin acetate is the acetate form of a hypothalamic peptide that stimulates the release of follicle-stimulating hormone and luteinising hormone from the pituitary gland. It contains not less than 95.0 per cent and not more than the equivalent of 102.0 per cent of the peptide $C_{55}H_{75}N_{17}O_{13}$, calculated with reference to the anhydrous, acetic acid-free substance. It is obtained by chemical synthesis.

CHARACTERS

A white or slightly yellowish powder, soluble in water and in a 1 per cent *V/V* solution of glacial acetic acid, sparingly soluble in methanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay. The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

Use the test solution and reference solution (a) prepared under Assay.

Apply to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 6 volumes of *glacial acetic acid R*, 14 volumes of *water R*, 45 volumes of *methanol R* and 60 volumes of *methylene chloride R*. Allow the plate to dry in air for 5 min. At the bottom of a chromatographic tank, place an evaporating dish containing a mixture of 10 mL of a 50 g/L solution of *potassium permanganate R* and 3 mL of *hydrochloric acid R*, close the tank and allow to stand. Place the dried plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 2 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application no longer gives a blue colour with 0.05 mL of *potassium iodide and starch solution R*. Spray with *potassium iodide and starch solution R*. The principal spot in the chromatogram obtained with the test solution corresponds in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution. A 10 g/L solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 10.0 mg in 1.0 mL of a 1 per cent *V/V* solution of *glacial acetic acid R*. The specific optical rotation is –54 to –66, calculated on the basis of the peptide content as determined in the assay.

Absorbance (*2.2.25*). Dissolve 10.0 mg in *water R* and dilute to 100.0 mL with the same solvent. The absorbance, determined at the maximum at 278 nm, corrected to a 10 mg/100 mL solution on the basis of the peptide content determined in the assay, is 0.55 to 0.61.

Amino acids. Examine by means of an amino-acid analyser. Standardise the apparatus with a mixture containing equimolar amounts of ammonia, glycine and the L-form of the following amino acids:

lysine	threonine	alanine	leucine
histidine	serine	valine	tyrosine
arginine	glutamic acid	methionine	phenylalanine
aspartic acid	proline	isoleucine	

together with half the equimolar amount of L-cystine. For the validation of the method, an appropriate internal standard, such as *DL-norleucine R*, is used.

Test solution. Place 1.0 mg of the substance to be examined in a rigorously cleaned hard-glass tube 100 mm long and 6 mm in internal diameter. Add a suitable amount of a 50 per cent *V/V* solution of *hydrochloric acid R*. Immerse the tube in a freezing mixture at –5 °C, reduce the pressure to below 133 Pa and seal. Heat at 110 °C to 115 °C for 16 h. Cool, open the tube, transfer the contents to a 10 mL flask with the aid of five quantities, each of 0.2 mL, of *water R* and evaporate to dryness over *potassium hydroxide R* under reduced pressure. Take up the residue in *water R* and evaporate to dryness over *potassium hydroxide R* under reduced pressure; repeat these operations once. Take up the residue in a buffer solution suitable for